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金缕梅科银缕梅属与帕罗堤属的亲缘关系——核糖体 DNA ITS 序列证据*

[1]李建华** [1] A. Linn Bogle [2] Anita S. Klein [3]潘开玉

CLOSE RELATIONSHIP BETWEEN *SHANIODENDRON* AND *PARROTIA* (HAMAMELIDACEAE), EVIDENCE FROM ITS SEQUENCES OF NUCLEAR RIBOSOMAL DNA

[1] Li Jian-hua [1] A. Linn Bogle [2] Anita S. Klein [3] Pan Kai-yu
[1](*Department of Plant Biology*, *University of New Hampshire*, Durham, NH 03824, USA)
[2](*Department of Biochemistry and Molecular Biology*, *University of New Hampshire*, Durham, NH 03824, USA)
[3](*Laboratory of Systematic & Evolutionary Botany*, *Institute of Botany*, *Chinese Academy of Sciences*, Beijing 100093, China)

Abstract The recently described genus *Shaniodendron* (Chang) Deng, Wei et Wang (Hamamelidoideae, Hamamelidaceae) is monotypic, narrowly distributed in eastern China, and on the verge of extinction. Morphological analysis puts this genus in the Fothergilleae *sensu* Harms, and implies that *Shaniodendron* is closely related to *Parrotia* C. A. Mey. In this study, sequences of the internal transcribed spacers of nuclear ribosomal DNA were analyzed for apetalous genera of the Hamamelidoideae to further evaluate the systematic relationships of *Shaniodendron*. The ITS-based phylogeny supported the combination of the Distylieae and the Fothergilleae, thus recognizing the Fothergilleae *sensu* Endress. It also substantiated the close relationship of *Shaniodendron* and *Parrotia*. The estimated divergence time of *Shaniodendron* and *Parrotia* was the late Miocene, which agreed with the fossil record.

Key words Hamamelidaceae; *Shaniodendron*; Phylogeny; nrDNA ITS

Shaniodendron (Chang)Deng, Wei et Wang is the most recently described genus in the Hamamelidaceae(Deng *et al*., 1992a). This monotypic genus was originally recognized as *Hamamelis subaequalis* by H. T. Chang (1979, 1960). It is endemic to East China, and found only at the eastern ranges of the Dabie mountains and the northern parts of the Tianmu mountains(Hao *et al*., 1996; Deng *et al*., 1992a, b;). Because of its rarity, *Shaniodendron* has been added to the list of the most endangered plant species in China(Deng *et al*.,

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** Responsible for correspondence(Telephone: 603-862-3209; Fax: 603-862-4757; e-mail: jli@christa.unh.edu).

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1992a, 1992b).

The diagnostic attributes of this genus are as follows: Deciduous trees up to 4～5 m. Leaves simple, broadly obovate; stipules two, lanceolate, caducous. Inflorescence capitate, 3～6 flowered. Flowers bisexual or staminate, precocious; hypanthium tube shallowly cupulate; petals absent; stamens 5～15, pendulous; anthers tetrasporangiate, dehiscing by simple slits; ovary semi-inferior, bicarpellate. Capsule woody, globose; Seeds one in each locule, brownish and glossy(Hao *et al*., 1996; Deng *et al*., 1992a;).

It seems reasonable to place *Shaniodendron* in the apetalous group of the Hamamelidoideae due to its lack of petals(Deng *et al*., 1992a). In terms of the detailed relationships of *Shaniodendron* with the other apetalous Hamamelidoideae, Deng *et al*.(1992a) suggested that this genus was more closely related to the Fothergilleae(incl. *Fothergilla* Murray, *Parrotia* C. A. Mey., *Parrotiopsis* Schneider) than to the Distylieae(incl. *Distylium* Sieb. et Zucc., *Matudaea* Lundell, *Molinadendron* Endress, and *Sycopsis* Oliv. (see Harms, 1930 and Endress, 1989a, b). Recently conducted comparative studies of floral morphology(Hao *et al*., 1996) and wood anatomy(Fang and Deng, 1996) support the placement by Deng *et al*.(1992a) of *Shaniodendron* in the Fothergilleae. The study of floral morphology, however, leads to the conclusion that *Shaniodendron* is most closely related to *Parrotia*(Hao *et al*., 1996).

Sequences of the Internal Transcribed Spacers(ITS) of nuclear ribosomal DNA have been almost routinely applied to phylogenetic studies of angiosperms at specific and generic levels (Baldwin *et al*., 1995; Campbell *et al*., 1995; Suh *et al*., 1993). Furthermore, our recent study has also shown that ITS data are potentially informative in resolving intergeneric relationships in the Hamamelidaceae(Li *et al*., 1996). Therefore, we chose ITS sequences as phylogenetic markers to evaluate the natural relationships of *Shaniodendron* with other members of the apetalous Hamamelidoideae.

1 Materials and methods

Materials of eight species, representing seven of the nine genera of Fothergilleae *sensu* Endress, was used in this study. However, *Matudaea* and *Molinadendron*, which Endress (1989b) included in his Fothergilleae, were not included in this analysis as our studies have suggested that these two genera do not belong to the Fothergilleae(unpublished). Three species of *Corylopsis* Sieb. et Zucc.(Corylopsideae) were used as the outgroup. Table 1 lists the species sampled for this study and their sequence accession numbers in the GenBank.

Total genomic DNAs were extracted from fresh leaves or buds following the protocol of Doyle and Doyle(1987). Polymerase Chain Reaction^{TM}(PCR) was conducted in 0.2 ml thin-walled microcentrifuge tubes. Each 50 μl reaction included 50～100 ng DNA template, 5 μl of *Taq* extender buffer(Stratagene, CA), 4 μl of 2.5 μm dNTP, 4 units of *Taq* extender (Stratagene, CA) and *Taq* polymerase(Promega, WI), 1 μl of 20 μm primers, and an appropriate amount of UV-treated distilled water.

Prior to the PCR reaction, hot-start strategy was used(D′ aquila *et al*., 1991). *Taq* polymerase and *Taq* extender were added into the reaction tube at the end of the hot-start when the temperature was stabilized at 80 ℃. Then, 30 cycles of PCR reaction were started following the thermocycler program of 105 seconds at 45 ℃, 115 seconds at 72 ℃ and 30 seconds at 94 ℃. The last cycle ended at 72 ℃ for 15 minutes of extension. The PCR primers were universal ITS4 and ITS5 of White *et al*.(1990).

Table 1　Species used in the parsimony analysis(AA, Arnold Arboretum; WI, Woodlanders Inc., SC; UNH, University of New Hampshire; HU, Harvard University)

Species	Voucher	Location	GenBank accession number
Corylopsis pauciflora(CPA)	J. Li 01	AA	U65462
C. sinensis(CSI)	J. Li 02	AA	U65461
C. spicata(CSP)	J. Li 03	AA	U65463
Distylium myricoides(DIM)	A. L. Bogle	WI	U65464
D. racemosum(DIR)	A. L. Bogle	WI	U65465
Distyliopsis tutcheri(DOT)	A. L. Bogle	WI	AF019231-2
Fothergilla major(FOT)	J. Li 11	UNH campus	AF015425-6
Parrotia persica(PAR)	A. L. Bogle	WI	AF015443
Parrotiopsis jacquemontiana(PPS)	A. L. Bogle	HU	AF015442
Shaniodendron subaequale(SHA)	Y. Qiu	Jiangsu, China	AF015431-2
Sycopsis sinensis(SYC)	A. L. Bogle	WI	AF015433-4

The PCR products were loaded on 0.8% LMP(Low Melting Point) agarose gel along with ØX174 *Hae* Ⅲ DNA size markers and run for 2～3 hours at 40 volts in 0.5× TBE buffer. The band identified by comparison to the markers was then excised from the gel, liquified at 65 ℃, and treated with agarase(Sigma Chemical Co., St. Louis, MO) for 30 minutes at 37 ℃. This gel-purified PCR product was used directly as sequencing template. Sequencing reactions were carried out using Cycle Sequencing Kit, FS(catalog #: 402119) and following the manufacturer's protocols(Applied Biosystems, CA).

The sequencing primers were ITS2, ITS3, ITS4 and ITS5 of White *et al*.(1990). The cycle sequencing products were then separated on 6% polyacrylamide gel using an Automated Sequencer 373A(Applied Biosystems, CA) in the Sequencing Facility Center of the University of New Hampshire(UNH).

The chromatograms were analyzed using the SEQED program (Applied Biosystems, CA). Also, in order to assure correct base-calling, we overlapped sequences generated from adjacent primers of either the same or opposite directions. The boundaries of ITS-1 and ITS-

2 were determined by comparing sequences of the 3′ 18S, 5.8S and 5′ end of the 26S ribosomal genes of *Canella winterana*(GenBank accession number L03844).

The sequences were aligned using the MEGALIGN program of DNA* software packages(DNA* Inc. WI). The aligned sequences(Fig.3) were imported into the PAUP(Phylogenetic Analysis Using Parsimony) computer program (Swofford, 1993) to search for shortest trees using exhaustive search option. Two separate analyses were conducted using gaps as the fifth character state and as missing data. All characters and their states were unweighted and unordered. *Corylopsis* was used as the outgroup in the parsimony analysis since our phylogenetic analysis of the Hamamelidaceae based on morphological data suggested that *Corylopsis* was the sister taxon to the Fothergilleae *sensu* Endress(unpublished). The mean distance matrices were exported from the PAUP and were used for the analysis of divergence. Both decay analysis(Bremer, 1988; Donoghue *et al*., 1992) and 500 replicates of bootstrap analysis(Felsenstein, 1985) were carried out to test the level of clade support using the PAUP program.

2 Results

2.1 Sequence characteristics The sequences of ITS-1 in the Fothergilleae *sensu* Endress (excl. *Matudaea* and *Molinadendron*) were all 271 bases long, while those of ITS-2 ranged from 232 to 237 bases in length with an average of 235(Table 2). GC content in the taxa sampled was 57~66% and 62~69% in ITS-1 and ITS-2 respectively. The Chi-square test showed that there was no significant difference among the genera in terms of the distribution of base composition in both ITS-1 and ITS-2($P>0.9$).

The alignment required 17 indels, most of which were one base long, while two indels were two to three bases in length and were found only between the ingroup and the outgroup species(Fig.3). The number of sites obtained from the alignment was 278 for ITS-1 and 240 for ITS-2 due to the addition of gaps, and the potentially informative sites were 22.63% in ITS-1 and 13.52% in ITS-2. The higher percentage of informative sites in ITS-1 than ITS-2 resulted from the longer lenth and higher variability of ITS-1. The pairwise ITS sequence divergence among the ingroup genera ranged from 0.4% between *Distylium racemosum* and *Distylopsis tutcheri* to 8% between *Parrotiopsis jacquemontiana* and *Sycopsis sinensis* in ITS-1, and from 0.8% between *Distyliopsis tutcheri* and *Sycopsis sinensis* to 6% between *Distylium myricoides* and *Fothergilla major* in ITS-2 (Table 3).

2.2 Phylogenetic tree When gaps were treated as the fifth character state in the parsimony analysis, two shortest trees were obtained from the exhaustive search with a length of 185 steps, and the Consistency Index(CI) and Rescaled Consistency Index(RC) were 0.89 and 0.8 respectively. In the strict consensus tree(Fig. 1), *Fothergilla* was the most basal taxon followed by *Parrotiopsis*. The next clade was paraphyletic to *Parrotiopsis* and contained two subclades, one of which was a trichotomy of 2 species of *Distylium* and *Distyliopsis tutcheri*,

Table 2　Sequence characteristics of ITS-1 and ITS-2 in the taxa sampled for this study (Taxon acronyms as in Table 1)

Taxon	ITS-1						ITS-2					
	Length	A	G	T	C	G+C	Length	A	G	T	C	G+C
CAP	269	22.68	28.25	17.47	31.60	59.85	233	15.88	32.19	21.03	30.90	63.09
CSI	274	23.36	28.10	18.98	29.56	57.66	234	14.96	33.33	20.09	31.62	64.95
CSP	275	24.00	28.00	18.55	29.45	57.45	234	15.81	32.91	20.09	31.20	64.11
DIM	271	21.40	29.52	14.39	34.69	64.21	235	15.74	31.91	18.30	34.04	65.95
DIR	271	21.40	29.52	14.39	34.69	64.21	235	15.32	31.49	17.87	35.32	66.81
DOT	271	21.40	29.52	14.76	34.32	63.84	236	15.25	31.36	19.07	34.32	65.68
FOT	271	20.30	29.52	16.97	33.21	62.73	235	15.74	31.49	21.70	31.06	62.55
PAR	271	20.66	30.26	13.28	35.79	66.05	235	15.32	31.49	19.15	34.04	65.53
PAR	271	20.66	29.89	13.65	35.79	65.68	232	15.52	31.47	18.97	34.05	65.52
SHA	271	20.30	30.63	13.28	35.79	66.42	236	15.68	31.78	18.22	34.32	66.10
SYC	271	20.30	30.26	13.65	35.06	65.32	237	15.19	31.65	18.99	34.18	65.83
Mean	271.45	21.50	29.41	15.40	33.63	63.04	234.73	15.49	31.92	19.41	33.19	65.10
Maximum	275	24.00	30.63	18.98	35.79	66.42	237	15.88	33.33	21.70	35.32	68.65
Minimum	269	20.30	28.00	13.28	29.45	57.45	232	14.96	31.36	17.87	30.90	62.26

Table 3　Sequence divergence (%) of ITS-1 (below diagonal) and ITS-2 (above diagonal) between the taxa sampled (Taxon acronyms as in Table 1). The values are derived from pairwise distances calculated using PAUP program

Taxon	CPA	CSI	CSP	DIM	DIR	DOT	FOT	PAR	PPS	SHA	SYC
CAP	–	2.95	3.82	12.07	12.86	11.59	11.86	11.57	10.86	12.87	11.98
CSI	4.14	–	0.84	10.81	11.18	9.90	11.04	9.89	10.04	11.19	10.29
CSP	3.78	2.22	–	11.68	12.04	10.76	11.90	10.75	10.92	12.05	11.16
DIM	17.79	19.80	19.76	–	1.69	2.12	6.42	2.11	4.77	2.54	2.95
DIR	17.74	19.74	19.70	1.13	–	1.26	5.52	1.26	3.89	1.67	2.08
DOT	17.82	19.83	19.79	0.76	0.37	–	4.27	0.84	2.58	1.26	0.83
FOT	16.66	19.07	18.66	5.29	5.67	5.67	–	4.24	3.82	5.50	4.27
PAR	18.95	20.95	20.90	1.88	3.00	2.62	5.69	–	2.58	1.26	1.68
PPS	18.58	20.58	20.54	6.37	7.12	7.12	6.43	5.26	–	3.86	3.43
SHA	18.56	20.57	20.52	2.25	3.39	2.99	6.06	1.87	6.38	–	2.10
SYC	19.88	21.86	21.82	3.40	4.15	3.76	7.60	3.40	7.95	3.78	–

while the other consisted of *Sycopsis* and the branch of *Parrotia* and *Shaniodendron*. The analysis treating gaps as missing data generated six equally parsimonious trees of 152 steps long, and the CI and RC were 0.9 and 0.83 respectively. The strict consensus tree (Fig. 2) did not resolve the relationships of *Sycopsis*, *Parrotia* and *Shaniodendron*; however, *Distylium racemosum* was bound with *Distyliopsis* instead of *Distylium myricoides* in a clade.

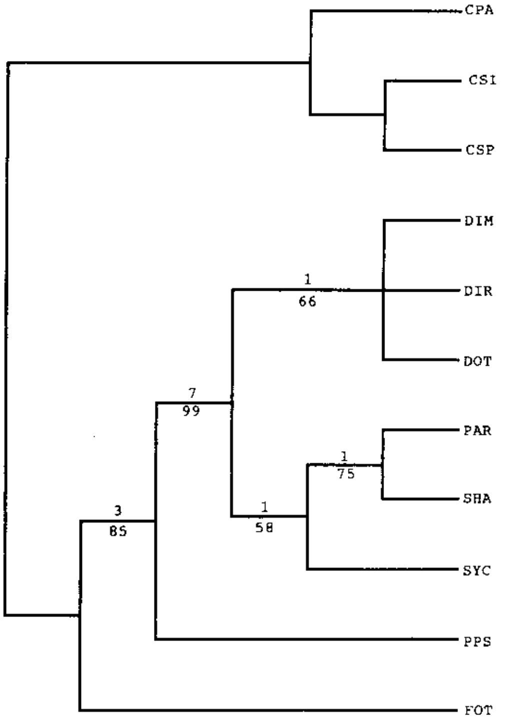

Fig.1 The strict consensus tree of the two equally most parsimonious trees of 185 steps generated using the exhaustive search, with gaps as the fifth character state. CI = 0.89 and RC = 0.8. Numbers above and below the branches represent decay indices and bootstrap percentages respectively. Taxon acronyms as in Table 1.

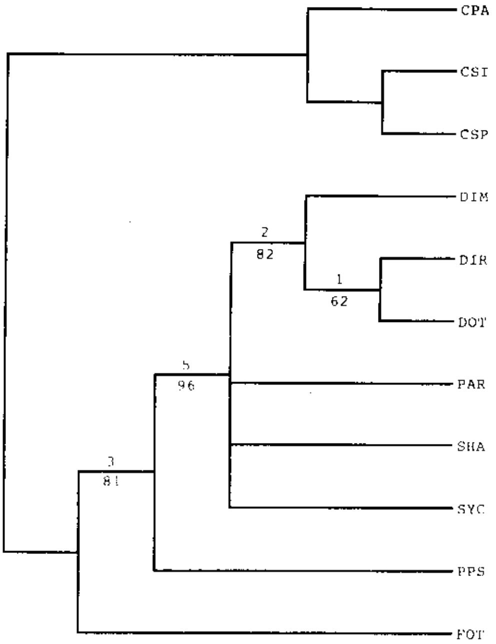

Fig. 2 The strict consensus tree of the six equally shortest trees of 152 steps produced using the exhaustive search, with gaps as missing data. CI = 0. 91 and RC = 0. 83. Numbers above and below branches are decay indices and bootstrap percentages respectively. Taxon acronyms as in Table 1.

2.3 Divergence time When the substitution rate of 5.5×10^{-10} bp per site per year was used, the estimated divergence time of *Parrotia* and *Shaniodendron* was about 14 my. The rationale of using this rate is discussed below.

3 Discussion

3.1 Sequence characteristics The lengths of the internal transcribed spacers vary from 187 to 298 for ITS-1 and from 187 to 252 for ITS-2; ITS-1 is generally longer and more divergent, thus contains more informative sites than ITS-2(review in Baldwin *et al*., 1995). The results of this study are concordant with the generalization.

3.2 Estimate of divergence time Molecular data, especially DNA sequences, have been applied to quantify genetic divergence for several disjunct taxa(Lee *et al*., 1996; Hoey and Parks, 1994, 1991; Wen and Zimmer, 1994; Wen and Jansen, 1992; Xiang *et al*., 1994; Parks and Wendel, 1990).

The sequence substitution rates of ITS vary considerably depending on the plant group and the method used to calibrate the time(based on fossil or geological history). A rate of substitution $3 \sim 5.5 \times 10^{-10}$ bases per site per year was estimated in Winteraceae(Suh *et al.*, 1993), 3.9×10^{-9} bases per site per year in *Dendroseries* (Asteraceae)(Sang *et al.*, 1994), and 7.8×10^{-9} bases per site per year in *Robinsonia* (Asteraceae)(Sang *et al.*, 1995). Suh *et al.* (1993) mentioned that their estimated substitution rates for the Winteraceae appeared relatively low. Lee *et al.* (1996) used a rate of 3.9×10^{-9} bases per year in *Dendroseries* to estimate divergence times for four pairs of disjunct taxa between eastern Asia and eastern North America: *Caulophyllum robustum-C. thalictroides*, *Menispermum dauricum-M. canadense*, *Penthorum chinense-P. sedoides* and *Phryma leptostachya* var. *asiatica-P. leptostachya* var. *leptostachya*. They found that the divergence times estimated using the substitution rate were roughly concordant with the fossil record, and with the previous hypothesis for the time of divergence between *Caulophyllum* and *Penthorum* (See reference for details). However, for *Menispermum*, the estimated time of divergence was much shorter than indicated by fossil record. Lee *et al.* (1996) gave two explanations for the low ITS substitution rate in *Menispermum*, one being concerted evolution and the other being long generation effect. The latter explanation seems to be more reasonable because previous studies have shown that long generation time results in low substitution rates(Baldwin *et al.*, 1995; Soltis and Soltis, 1995; Suh *et al.*, 1993), and concerted evolution generally leads to intraspecific homogenization and interspecific heterogeneity(Suh *et al.*, 1993; Dover, 1982).

Another factor that may affect ITS substitution rates is the antiquity of the taxon. For instance, the Winteraceae, one of the most primitive families of angiosperms, has much lower ITS substitution rates than the more advanced taxa of the Asteraceae such as Dendroseries and Robinsonia(Sang *et al.*, 1995, 1994; Suh *et al.*, 1993). Therefore, considering that both *Parrotia* and *Shaniodendron* are woody plants with a long generation time, and are evolutionarily close to, but more advanced than Magnolidae, we chose the high end of ITS substitution rates ($3 \sim 5.5 \times 10^{-10}$ base substitutions per site per year) estimated for the Winteraceae by Suh *et al.* (1993), which is 5.5×10^{-10} bases per site per year. Interestingly, when we applied 100 my as the separation time of Madagascar from the African continent (Harland *et al.*, 1990; Pielou, 1979; Shuster, 1976), the estimated substitution rate of ITS between two closely related genera of subtribe Dicoryphinae(Endress, 1989b), *Dicoryphe* Du Petit-Thouars (endemic to Madagascar and Comoro Islands) and *Trichocladus* Pers. (native to eastern and southern Africa)(Hamamelidaceae) is about 5.5×10^{-10} bases per site per year(unpublished). The estimated divergence time between *Parrotia* and *Shaniodendron* is about 14 my, which is in the late Miocene(Harland, 1990; Pielou, 1979). The fossil record indicates that *Parrotia* was distributed in both southeastern and western Asia in the middle Miocene(Huzioka, 1970; Stuchlik and Shatilova, 1987; Zhang and Lu, 1995), while *Shaniodendron* was present at least in northern China in the Miocene(Deng *et al.*, 1992a, b). Thus, the estimated divergence time between *Shaniodendron* and *Parrotia* is well within the range of the fossil record.

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          |-->ITS-1
Taxon             10        20        30        40        50        60        70        80
                   .         .         .         .         .         .         .         .
CPA       TCGAAACCTGCCCAGCAGAACGACCCGCGAACACGTAAAAA-TACAAGTGGGGGCGGGGGGGG----CATGCTCCTCTCA
CSI       TCGAAACCTGCCCAGCAGAACGACCCGCGAACACGTAAAAAATACAAGTGGGGGGGGGGGGGCA-TTCGTGCTCCTCTCA
CSP       TCGAAACCTGCCCAGCAGAACGACCCGCGAACACGTAAAAAATACAAGTGGGGGCGGGGGGGCAATCCGTGCTCCTCTCA
DIM       TCGAAACCTGCCCAGCAGAACGACCCGCGAACACGTATCAA-TGC-TACGGGGGCGAGGGG-AGGCATGCCCCCTCTCTC
DIR       TCGAAACCTGCCCAGCAGAACGACCCGCGAACACGTATCAA-TGCATAAGGGGGCGAGGGG-AGGCATGCCCCCTCTCTC
DOT       TCGAAACCTGCCCAGCAGAACGACCCGCGAACACGTATCAA-TGC-TAAGGGGGCGAGGGG-AGGCATGCCCCCTCTCTC
FOT       TCGAAACCTGCCCAGCAGAACGACCCGCGAACACGTATCAA-TGC-TATGGGGGCGAGGGGTGGGCATGCCCCCTCTCTC
PAR       TCGAAACCTGCCCAGCAGAACGACCCGCGAACACGTATCAA-TGC-TACGGGGGCGAGGGG-AGGCATGCCCCCTCTCTC
PPS       TCGAAACCTGCCCAGCAGAACGACCCGCGAACACGTATCAA-TGC-TACGGGGGCGGGGGG-AGGCATGCCCCCTCTCTC
SHA       TCGAAACCTGCCCAGCAGAACGACCCGCGAACACGTATCAA-TGC-TGCGGGGGCGAGGGG-AGGCATGCCCCCTCTCTC
SYC       TCGAAACCTGCCCAGBAGAACGACCCGCGAACACGTNTCAA-TGC-TACGGGGGCGAGGGG-AGGCATGCCCCCTCTCTC

                  90       100       110       120       130       140       150       160
                   .         .         .         .         .         .         .         .
CPA       CCCCCAAACTCGAGACGCGCTCGGTGCTCGATCAGCAACGCGTGCC-TCGCGCACGGGAAGCTTCGGTG-CCGTCGTGCT
CSI       CCCCCAAAATCGGGACGCGCTCGGTGCTCGATTAGAAACGCGTGCC-TCGTGCACGGGAAGCTTCGGTG-CCGTCGTGCT
CSP       CCCCCAAAATCGGGACGCGCTCGGTGCTCGATTAGCAACGCGTGCC-TCGTGAACGGGAAGCTTCGGTG-CCGTCGTGCT
DIM       CCCC-AAAGTCGGGACGCGCTCGGTGCTCGATCCGCCAACCGTGCC-CCGTGCACGGGAAGCACCGGCA-CCGGCGTGCG
DIR       CCCC-ATAGTCGGGACGCGCTCGGTGCTCGATCCGCCAACCGTGCC-CCGTGCACGGGAAGCACCGGCA-CCGGCGTGCG
DOT       CCCC-ATAGTCGGGACGCGCTCGGTGCTCGATCCGCCAACCGTGCCACCGTGCACGGGAAGCACCGGCA-CCGGCGTGCG
FOT       CCCC-AAAGTCGGGACGCGCTCGGTGCTCGATCCGCCAACCGTGCC-CCGTGCGCGGGAAGCATCGGCA-CCGGCGTGCG
PAR       CCCCCAAAGTCGGGACGCGCTCGGTGCTCGATCCGCCAACCGCGCCAC-GTGCGCGGGAGGCACCGGCA-CCGGCGTGCG
PPS       CCCC-AAAGTCGGGACGCGCTCGGTGCTCGACTCGCCAAACGTGCC-CCGCGCGCGGGAGGCATCGGCA-CCGGCGTGCG
SHA       CCCCCAAAGTCGGGACGCGCTCGGTGCTCGATCCGCCGACCGTGCCAC-GTGCGCGGGAAGCACCGGCA-CCGGCGTGCG
SYC       CCCC-AGAGCCGGGACGCGCTCGGTGCTCGATCCGCCAACCGTGCCAC-GTGCCCGGGAAACCCCGGGAACCGGCGTGGG


    (Continued)

                 170       180       190       200       210       220       230       240
                   .         .         .         .         .         .         .         .
CPA       CTCGATCTCACAACGAACCCCGGCGCAAAACGCGTCAAGGAATCTAAA-TGCAAGAGCGTGCTCCCATCCCCCGGTCACG
CSI       CTCGATCTCACAACGAACCCCGGCCCAAAACGCGTCAAGGAATCTAAAATGCAAGAGCGTGCTCCCATCGCCCGGTCACG
CSP       CTCGATCTCACAACGAACCCCGGCGCAAAACGCGTCAAGGAATCTAAAATGAAAGAGCGTGCTCCCATCGCCCGGTCACG
DIM       CTCGGCATCACAACGAACCCCGGCGCAAATCGCGTCAAGGAAAACGAAACGCAAGAGCTTGCTCCCATCCCCCGGTCTCG
DIR       CTCGGCATCACAACGAACCCCGGCGCAAACCGCGTCAAGGAAA-CGAAACGCAAGAGCTTGCTCCCATCCCCCGGTCTCG
DOT       CTCGGCATCACAACGAACCCCGGCGCAAATCGCGTCAAGGAAA-CGAAACGCAAGAGCTTGCTCCCATCCCCCGGTCTCG
FOT       CTCGATATCACAACGAACCCCGGCGCAAAACGCGTCAAGGAAA-CGCAACGTAAGAGTCTGCTCCCATCCCCCGGTCTCG
PAR       CTCGGCATCACAACGAACCCCGGCGCAAATCGCGTCAAGGAAA-CGAAACGCAAGAGCCCGCTCCCATCCCCCGGTCTCG
PPS       CTCGATATCACAACGAACCCCGGCGCAAAACGCGTCAAGGAAA-CGCAACGCAAGAGCCCGCTCCCATCCCCCGGTCTCG
SHA       CCCGGCATCACAACGAACCCCGGCGCAAATCGCGTCAAGGAAA-CGAAACGCAAGAGCCCGCTCCCATCCCCCGGTCTCG
SYC       CTCGGCATCACAACGAACCCCGGCGCAAATCGCGTCAAGGAAA-CGAAACGCAAGAGCCCGCTCCCATCCCCCGGTCTCG

                 250       260       270                 10        20        30        40
                                                 |-->ITS-2
                   .         .         .         .        .         .         .         .
CPA       GGTTGTGGTGGGGGCAATGTCATCTTCGATATTATT-C CGTATCGCGTCGCC-CCCCAA--CCCCGCTCACGTCCAAC
CSI       GGTTTTGGTGGGGGCAATGTCATCTTCGATATTATT-C CGTATCGCGTCGCC-CCCCAA--CCTCGCCCACGTCCAAC
CSP       GGTTTTGGTGGGGGCAATGTCATCTTCGATATTATT-C CGTATCGCGTCGCC-CCCCAA--CCTCGCCCACATCCAAC
DIM       GGTCGTGGTGGGGGCGATGGCATCTTCGATATTAT-AC CGTATCGCGTCGCCACCCCAA-CCCCCGCACACGCATGCC
DIR       GGTCGTGGTGGGGGCGATGGCATCTTCGATATTAT-AC CGTACCGCGTCGCCACCCCAA-CCCCCGCCCACGCATGCC
DOT       GGTCGTGGTGGGGGCGATGGCATCTTCGATATTAT-AC CGTATCGCGTCGCCACCCCAA-CCCCCGCCCACGTATGCC
FOT       GGTTGTGGTGGGGGCATTGGCATCTTCGATATTATT-C CGTATTGCGTCGCCACCCCAA-CCCCCGTCCACGTATATC
PAR       GGTCGTGGTGGGGGCGATGGCATCTTCGATATTAT-AC CGTATCGCGTCGCCACCCCAA-CCCCCGCCCACGCATGCC
PPS       GGTCGCGTTGGGGGCGATGGCATCTTCGATACTATTAC CGTATCGCGTCGCCACCC-AA-CCCCCGCCCACGTACACC
SHA       GGTCGTGGTGGGGGCGATGGCATCTTCGATATTAT-AC CGTATCGCGTCGCCACCCCAAACCCCCGCCCACGCATGCC
SYC       GGTCGTGGTGGGGGCGATGGCATCTTCGATATTAT-TC CGTATCGCGTCGCCACCCCAA-CCCCCGGCCACGTATGCC
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                50        60        70        80        90       100       110       120
                 .         .         .         .         .         .         .         .
CPA     GTGGTGCGGGGCATTATGCGGGGATGGATATTGGCCTCCCGTGCGCTGTGCTGCGGTTGGCCTAAAAGCGAGCCCCGGGC
CSI     GTGGTGCGGGGCATTATGCGGGGGCGGATATTGGCCTCCCGTGCGCTGCGCTGCGGTTGGCCTAAAAGCGAGCCCCGGGC
CSP     GTGGTGCGGGGCATTATGCGGGGGCGGATATTGGCCTCCCGTGCGATGCGCTGCGGTTGGCCTAAAAGCGAGCCCCGGGC
DIM     GTGATGCGGGGCGTACTGTGGGGGCGGATATTGGCCTCCCGTGCGCTTCGCCGCGGTTGGCCTAAAAGCGAGCCCCGGGC
DIR     GTGATGCGGGGCGTCCTGTGGGGGCGGATATTGGCCTCCCGTGCACTTCGCCGCGGTTGGCCTAAAAGCGAGCCCCGGGC
DIT     GTGATGCGGGGCGTCCTGTGGGGGCGGATATTGGCCTCCCGTGCACTTCGCCGCGGTTGGCCTAAAAGCGAGCCCCGGGC
FOT     GTGCTGCGGGGCGTCATGTGGGGGCGGATATTGGCCTCCCGTGCACTTCGCCGCGGTTGGCCTAAAAGCGAGCCCCGGGC
PAR     GTGATGCGGGGCGTCCTGTGGGGGCGGATATTGGCCTCCCGTGCACTTCGCCGCGGTTGGCCTAAAAGCGAGCCCCGGGC
PPS     GTGCCGCGGGGCGTCTTGTGGGGGCGGATATTGGCCTCCCGTGCACTTCGCCGCGGTTGGCCTAAAAGCGAGCCCCGGGC
SHA     GTGATGCGGGGGGTCCTGTGGGGGCGGATATTGGCCTCCCGTGCACTTCGCCGCGGTTGGCCTAAAAGCGAGCCCCGGGC
SYC     GTGATGCGGGGCGTCCTGTGGGGGCGGATATTGGCCTCCCGTGCACTTCGCCGCGGTTGGCCTAAAAGCGAGCCCCGGGC

               130       140       150       160       170       180       190       200
                 .         .         .         .         .         .         .         .
CPA     -GAC-AGAACGTCACGATAAGTGGTGGTTTATAAGCCCCGACCCTTGCAGTGTCGTGTCGTGCGTTGCACTGTCGCTCGT
CSI     -GAC-AGAACGTCACGATAAGTGGTGGTTTATAAGCCCCGACCCTTGCAGTGTCGTGTCGTGCGTTGCACTGTCGCTCGT
CSP     -GAC-AGAACGTCACGATAAGTGGTGGTTTATAAGCCCCGACCCTTGCAGTGTCGTGTCGTGCGTTGCACTGTCGCTCGT
DIM     -GAC-AGAACGTCACGATAAGTGGTGGTTGATAAGCCCCGGCCCTTCAAGTGTCATGTCGTGCGTTGCCCTGTCGCCCGT
DIR     -GAC-AGAACGTCACGATAAGTGGTGGTTGATAAGCCCCGGCCCTTCAAGTGTCATGTCGTGCGTTGCCCTGTCGCCCGT
DOT     -GACCAGAACGTCACGATAAGTGGTGGTTGATAAGCCCCGGCCCTTCAAGTGTCATGTCGTGCGTTGCCCTGTCGCCCGT
FOT     -GAC-AGAACGTCACGATAAGTGGTGGTTGATAAGCCCCGGCCGTTCAAGTGTCATGTCGTGCGTTGCCCTGTCGCTTGT
PAR     -GAC-AGAACGTCACGATAAGTGGTGGTTGATAAGCCCCGGCCCTTCAAGTGTCATGTCGTGCGTTGCCCTGTCGCTCGT
PPS     -GAC-AGAACGTCACGATAAGTGGTGGT-GATAAGCCCCGGCCCTTCAAGTGTCATGTCGTGCGTTGCC-TGTCGCTCGT
SHA     -GAC-AGAACGTCACGATAAGTGGTGGTTGATAAGCCCCGGCCCTTCAAGTGTCATGTCGTGCGTTGCCCTGTCGCCCGC
SYC     CGACCAGAACGTCACGATAAGTGGTGGTTGATAAGCCCCGGCCCTTCAAGTGTCATGTCGTGCGTTGCCCTGTCGCCCGT

                           (Continued)

               210       220       230       240
                 .         .         .         .
CPA     T-GGAGCTA-TGAGACCCCGATGCGCCGTACCGACGGTGC
CSI     T-GGAGCTAATGAGACCCCGATGCGCCGTGCCGACGGTGC
CSP     T-GGAGCTAATGAGACCCCGATGCGCCGTGCCGACGGTGC
DIM     CCGGAGCTA-CGAGACCC-GATGCGTCGTCCCGACGATGC
DIR     C-GGAGCTA-CGAGACCCCGATGCGTCGTCCCGACGATGC
DOT     T-GGAGCTA-CGAGACCCCGATGCGTCGTCCCGACGATGC
FOT     T-GGAGCTA-CGAGACCCCGATGCGTCGTTCCGACGATGC
PAR     T-GGAGCTA-CGAGACCCCGATGCGTCGTCCCGACGATGC
PPS     T-GGAGCTA-CGAGACCCCGATGCGTCGTCCCGACGATGC
SHA     T-GGAGCTA-CGAGACCCCGATGCGTCGTCCCGACGATGC
SYC     T-GGAGCTA-CGAGACCCCGATGCGTCGTCCCGACGATGC
```

Fig. 3　The aligned ITS sequences of the taxa used in this analysis. Taxa acronyms as in Table 1.

3.3　Phylogenetic relationships　The apetalous Hamamelidoideae (excl. Matudaea and *Molinadendron*) is a monophyletic group (our unpublished data) and has been divided into two tribes, the Distylieae, including *Distylium*, *Distyliopsis* and *Sycopsis*, and the Fothergilleae, consisting of *Fothergi-lla*, *Parrotia* and *Parrotiopsis* (Endress, 1989a, b; Chang, 1979; Bogle, 1970; Harms, 1930). The morphological attributes delimiting the two tribes are persistent leaves and andromonoecious flowers in the Distylieae versus deciduous leaves and bisexual flowers in the Fothergilleae (Endress, 1989a, b). However, on the basis of the discovery of some spontaneous hybrids between *Sycopsis* and *Parrotia* in the botanical garden at Zurich (× *Sycoparrotia*, Endress and Anliker, 1968), Endress (1989a, b) suggested that the Distyleae and the Fothergilleae be combined into one tribe, the Fothergilleae *sensu* Endress. In the ITS-based phylogenies (Fig. 1, 2), neither the Distylieae nor the Fothergilleae formed a monophyletic clade, which supports combining the Distylieae and the Fothergilleae, and recognizing the Fothergilleae *sensu* Endress.

Fothergilla is rather unique morphologically in the Hamamelidaceae as well as in the Fothergilleae. It is characterized by centrifugal stamen development, greatly elongated, club-shaped, whitish and showy filaments, and very small anthers. In the ITS phylogeny (Fig. 1, 2), *Fothergilla* formed its own clade and was located in the basal position of the Fothergilleae *sensu* Endress, suggesting that *Fothergilla* is isolated from the remaining genera.

Parrotiopsis is the other genus in the Hamamelidaceae that lacks petals but uses modified organs (showy bracts subtending the inflorescence) to attract pollinators (*Fothergilla* has showy filaments). This characteristic, together with the cellular type of endosperm formation, is peculiar in the Hamamelidaceae (Kapil and Kaul, 1972; Bogle, 1970). In Figures 1 and 2, *Parrotiopsis* was the first taxon following *Fothergilla* and formed its own clade. The relationships of *Parrotiopsis* and the remaining genera were relatively strongly supported by about 80% of bootstrap and a decay of three steps. Despite its unique whitish and showy bracts, *Parrotiopsis* shares many morphological characteristics with *Parrotia*, *Distylium*, *Shaniodendron* and *Sycopsis*, including the simple slit type of anther dehiscence, somewhat elongated anthers, and slightly distinct connective protrusions (Bogle, 1970; Endress, 1989a).

The strongest support from the ITS data (a bootstrap of 99% and a decay of seven steps) is offered to the clade containing two weakly allied branches, one of which consists of *Distylium* and *Distyliopsis*, while the other is composed of *Sycopsis*, *Parrotia* and *Shaniodendron* (Fig. 1). The relationships among species of *Distylium* and *Distyliopsis* are not resolved in this phylogeny, but when gaps are treated as missing data, *Distyliopsis* is nested within species of *Distylium* (Fig. 2). This pattern, in the first place, suggests a close relationship of *Distyliopsis* and *Distylium*, but on the other hand, casts doubt on the generic status of *Distyliopsis*. However, there is only one species of *Distyliopsis* available for this study, thus the results regarding the generic status of *Distyliopsis* are not conclusive. An analysis including more species is needed to get a better picture of the relationships between *Distylium* and *Distyliopsis*.

Sycopsis, *Parrotia* and *Shaniodendron* form a polytomous clade in the tree generated from the analysis using gaps as missing data (Fig. 2), indicating their close relationships to one another. The parsimony analysis treating gaps as the fifth character suggests a closer relationship of *Parrotia* to *Shaniodendron* than to *Sycopsis* (Fig. 1), as proposed by the recent studies of floral morphology and wood anatomy of *Shaniodendron* (Hao *et al*., 1996;

Fang and Deng, 1996).

In summary, the ITS-based phylogeny supports the combination of the Distylieae and the Fothergilleae and recognizes the Fothergilleae sensu Endress. This study also substantiates the close relationship of *Shaniodendron* and *Parrotia*. However, the relatively weak support indices for their clade indicate an early divergence between the two taxa. The estimated divergence time(late Miocene) of *Shaniodendron* and *Parrotia* agrees with the fossil record.

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摘要　本文对金缕梅科弗特吉族(Molinadendron 和 Matudaea 除外)的细胞核核糖体 DNA ITS 片段进行了序列分析,在此基础上对该族的演化关系、尤其是中国特有属银缕梅属的系统位置进行了最简约分支分析。基本结果如下:其一,蚊母族和狭义的弗特吉族均不形成自己的单系分支,因此,支持 Endress 的处理,即合并两族为广义的弗特吉族;其二,银缕梅属同帕罗堤属有着密切的亲缘关系,支持形态学和解剖学结论;其三,根据该片段核苷酸序列的演化速率推算出的银缕梅属和帕罗堤属属间分化时间为晚中新世,此结论大致与化石记录相符。

关键词　金缕梅科; 银缕梅属; 系统发育; nrDNA ITS